and has a much shorter M3-M4 loop. To determine whether (or not) these segments are crucial for the function of a eukaryotic acetylcholine-glutamate Cys-loop chimeric receptor (α7-GluClβR), we deleted those segments of the chimera that are missing in GLIC. Ligand-binding assays performed on transfected living cells indicate that chimeras lacking most of the M3-M4 loop can readily bind 3H-α-bungarotoxin (a competitive antagonist) and nicotine (an agonist). These deletion chimeras were visualized on the cell surface by confocal microscopy using rhodaminylated α-bungarotoxin and specific antibodies. In addition, chimeras lacking the M3-M4 loop display ACh-induced currents with unchanged EC50, Hill coefficient and ionic selectivity. In contrast, chimeras lacking the N-terminal helical segment do not bind 3H-α-bungarotoxin. However, these N-terminus-truncated receptors migrate as non-degraded proteins in SDS PAGE and are readily visualized on the surface of transfected cells with specific anti-HA tag antibodies. Electrophysiological experiments are currently performed to determine whether (or not) acetylcholine, nicotine or protons activate the N-terminus truncated chimeras. Supported by the Wolfson Family Foundation and the Israel Science Foundation.

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Number of Extracellular-Transmembrane Interfaces Required for Activation of Homomeric Cys-Loop Receptors

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Each subunit in a homo-pentameric Cys-loop receptor contains a specialized transduction zone located at the extracellular-transmembrane interface that links the ligand binding domain to the ion conductive channel. To determine the contribution of each transduction zone to stability of the open channel, we constructed a subunit with both a disabled transduction zone and a reporter mutation that alters unitary conductance, and co-expressed mutant and normal subunits. The resulting receptors show single channel current amplitudes that are quantized according to the number of reporter mutations per receptor, allowing correlation of mean open time with the number of intact transduction zones. We find that each transduction zone contributes an equal increment to the stability of the open channel. However by combining subunits with either disabled agonist binding sites or transduction zones, we find that although each binding site is formed by a pair of subunits, detectable channel opening requires an intact transduction zone in both subunits. By manipulating the numbers and locations of transduction zones and binding sites, we find that a transduction zone in a subunit at an inactive binding site can still stabilize the open channel. The findings show that although the agonist binding sites and transduction zones contribute allosterically to open channel stability, their stoichiometry and positioning requirements are distinct.

1509-Pos Board B419

Identification of the Binding Site for the Anthelmintic Drug Ivermectin in Cys-Loop Receptors

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Ivermectin (IVM) is an antiparasitic drug widely used in veterinary medicine, mainly in cattle, to kill intestinal worms. Additionally, it is used to treat human parasitic diseases like Onchocerciasis (river blindness). The major target of IVM is a heteropentameric glutamate-gated chloride channel (GluClα/β receptor), which is unique to invertebrates and belongs to the Cys-loop receptor superfamily. Nanomolar concentrations of IVM irreversibly activate the native GluClα/βR, thereby causing sustained hyperpolarization, suppression of nervous impulses, paralysis and death of the nematode. Little is known about the IVM binding site(s). We first studied a chimeric α7-GluClβ receptor that consists of the ligand-binding domain (LigBD) of the α7 nicotinic acetylcholine receptor (α7-nAChR) and the channel domain of a homopentameric GluClβR. IVM was expected to increase the chimera's activity since the activity of the α7-nAChR is enhanced by IVM and the homopentameric GluClβR is irresponsive to IVM. Surprisingly, IVM strongly inhibited the chimeric α7-GluClβR suggesting that IVM does not bind exclusively to either of the domains, but binds somewhere at the interface between the LigBD and the pore domain. Next, we explored the natural target of IVM, the GluClα/βR. Since the α-subunit is known to be responsible for IVM binding by the native GluClα/βR, we replaced the β8β9 and Cys loops of the β-subunit by the homologous loops of the α-subunit. These two loops reach the interface between the LigBD and the pore domain. Our results show that the aforementioned loop swapping significantly enhances receptor activation by IVM. Furthermore, site-specific mutagenesis suggests that IVM binds at the interface between the LigBD and the pore domain, in a crevice between the β8β9 loop and the Cys loop of two adjacent subunits. Supported by the Wolfson Foundation and the Israel Science Foundation.

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Transmembrane Domain Packing in Cys-Loop Receptors: What Can we Learn from Prokaryotes?

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The significance of large gaps in protein density observed in the cryo-EM structure (2BG9) of the nicotinic acetylcholine receptor (nAChR) transmembrane domain remains a pressing question. Although it was originally proposed that the gaps were filled with water, water-filled pockets are not stable under simulation, causing collapse of the transmembrane domain. Alternative explanations include the possibility that the gaps are filled with cholesterol or other lipid, as proposed by our group, or that the gaps are an artifact of the experimental method. Recent crystal structures of prokaryotic homologues, including GLIC, do not include such gaps, supporting the latter scenario. However, here we show through homology modeling that models of the nAChR based on GLIC still retain low protein density in the extracellular half of the TM domain, due to consistently smaller residue volume in that region of the nAChR compared to GLIC. Simulations of this model over the 100ns time scale also display collapse that increases amino acid density in this region of the protein, although for this model collapse largely proceeds by significant widening of the pore rather than reduction in the overall footprint. An analysis of residue volume, determined from sequence, is presented across identified prokaryotic, eukaryotic anionic, and eukaryotic cationic members of the pentameric ligand-gated ion channels family, demonstrating that residue volume has been consistently lost as channels become less primitive, and that a tightly-packed nAChR would require a substantially tighter packing of backbone helices than what is observed in prokaryotic channels.

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Oligomeric Structure of Muscarinic and Adrenergic Receptors in Live Cells

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We have determined the oligomeric size and configuration of fluorophore-tagged M_1, M_2, β_1, and β_2 receptors in the plasma membrane of Chinese hamster ovary (CHO) cells by examining the distribution of FRET efficiencies measured at the level of single pixels. The receptors were fused at the *N*-terminus to enhanced green or yellow fluorescent protein, and complementary pairs were co-expressed at different ratios of donor to acceptor (*i.e.*, $eGFP^2$-M_1 and eYFP-M_1, $eGFP^2$-M_2 and eYFP-M_2, $eGFP^2$-β_1 and eYFP-β_1, or $eGFP^2$-β_2 and eYFP-β_2). Pixel-level emission spectra were recorded from images captured in a single plane; the relative contribution of each fluorophore then was determined by spectral deconvolution and used to calculate the corresponding apparent FRET efficiency. The distributions of efficiencies from 20 cells were analyzed in concert as a sum of Gaussians, with the number of components (n) and the relationships among the means taken as predicted for a dimer (n = 1), a triangular trimer (n = 2), and a tetramer configured as a square (n = 3) and a rhombus (n = 5). Distributions from each of the four receptors required 5 Gaussians, the means of which were related in the manner predicted for a rhombus. The inverse agonists atropine and timolol were without effect on the number of components or the relationship among the means detected for the M_2 muscarinic receptor and the β_2 adrenergic receptor, respectively. Homo-oligomers of the M_1, M_2, β_1, and β_2 receptors therefore appear to be rhombic tetramers in CHO cells. The configuration is unaffected by inverse agonists, at least in the case of the M_2 and β_2 receptors.

Mechanosensitive Channels

1512-Pos Board B422

MscI Channels as Nanovales for the Controlled Release of Liposome-Encapsulated Compounds

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MscL acts as an osmotically-activated nanovalve, allowing bacteria to respond to hypo-osmotic stress by opening nanometer-size channel pores. The underlying mechanism of channel activation by membrane tension has been obtained for MscL channels reconstituted into artificial liposomes using patch clamp, EPR and FRET spectroscopy in combination with computational modeling of channel dynamics during channel opening. Given the large size of the MscL pore (>25 Å), we have investigated its suitability for use as a nanovalve enabling controlled release of liposome-encapsulated compounds. Liposomes present one of the major forms of particulate drug carriers and provide an excellent method of encapsulation of highly toxic drugs, for example. In this study we describe methods for generating small liposomes of uniform size